



**Karolinska  
Institutet**

**Department of Microbiology, Tumor and Cell Biology**

# **Chronic Immune Activation and Lymphocyte Apoptosis during HIV-1 Infection**

**AKADEMISK AVHANDLING**

som för avläggande av medicine doktorsexamen vid Karolinska  
Institutet offentligen försvaras i Föreläsningssalen vid  
Institutionen för Mikrobiologi, Tumör and Cellbiologi (MTC),  
Theorellsväg 1, Karolinska Institutet

**Fredagen den 17 februari, 2012, kl 09.00**

av

**Nicolas Ruffin**

MSc.

*Huvudhandledare:*

Prof Francesca Chiodi  
Karolinska Institutet  
Institutionen för Mikrobiologi,  
Tumör and Cellbiologi

*Bihandledare:*

Dr Bence Rethi  
Karolinska Institutet  
Institutionen för Mikrobiologi,  
Tumör and Cellbiologi

Prof Martin Cranage  
St. George's University of London  
Division of Clinical Sciences  
Centre for Infection & Immunity

*Fakultetsopponent:*

Prof Alan L. Landay  
Rush University Medical Center  
Department of Immunology/Microbiology

*Betygsnämnd:*

Docent Eva Sverremark-Ekström  
Stockholm University  
Wenner-Gren Institute  
Department of Immunology

Prof Markus Maeurer  
Karolinska Institutet  
Institutionen för Mikrobiologi,  
Tumör and Cellbiologi

Prof Anders Vahlne  
Karolinska Institutet  
Institutionen för laboratoriemedicin  
Avdelningen för klinisk mikrobiologi

**Stockholm 2012**

## ABSTRACT

HIV-1 infected individuals are subject to a chronic immune activation resulting from HIV-1 replication, microbial translocation, and lymphopenia. Despite the great advance of antiretroviral treatment (ART), the immune activation remains associated with poor immune reconstitution during HIV-1 infection. The overall aim of this PhD thesis is to contribute to a better understanding of the causes and consequences of immune activation, possibly leading to the design of improved therapy for HIV-1 infected individuals.

Premature senescence of T cells, as a consequence of immune activation, is thought to be associated with the increased levels of CD28<sup>+</sup> T cells during HIV-1 infection. In **Paper I**, the phenotype and functional properties of CD28<sup>+</sup> T cells from HIV-1 individuals naïve to treatment, under ART and uninfected controls were assessed. Despite displaying similar markers of senescence, and late differentiation, we found that whereas CD28<sup>+</sup> T cells from untreated patients are highly susceptible to both spontaneous and activation-induced apoptosis, the same T cell population from ART-treated patients showed an enhanced capacity to proliferate upon weak TCR stimulation. Importantly, apoptosis of CD28<sup>+</sup> T cells from untreated patients was correlated with HIV-1 viral load, and their decreased ability to proliferate was associated with a reduced IL-2 production. High levels of CD28<sup>+</sup> T cells during HIV-1 infection might result from the chronic immune activation, whereas their sustained levels despite ART, is likely to arise from their capacity to proliferate under weak TCR signaling. Furthermore, with a capacity to produce IFN- $\gamma$ , TNF and perforin, CD28<sup>+</sup> T cells from HIV-1 infected individuals might also contribute to the immune activation.

The mechanisms underlying the loss of memory B cells and the decline of serological memory during HIV-1 infection remain elusive. As microbial translocation and the associated immune activation have been shown to correlate with T cell depletion, we evaluated, in **Paper II**, the association between the serum levels of soluble CD14, a marker of microbial translocation, with the loss of resting memory B cells in HIV-1 infected individuals. Soluble CD14 levels were found to correlate with both the decline of resting memory B cells, and their increased expression of IL-21R. IL-21R expression on memory B cells was increased during HIV-1 infection, and also negatively correlated with the levels of circulating memory B cells. Notably, IL-21R positive memory B cells were more prone to apoptosis, measured by higher Annexin V staining and lower Bcl-2 expression, as compared to B cells lacking the receptor. Furthermore, TLR triggering by microbial products resulted in IL-21R expression on memory B cells *in vitro*. Our results identify a novel role for microbial translocation and the associated immune activation, contributing to the loss of memory B cells during HIV-1 infection.

Lymphopenic conditions are associated with increased IL-7. This cytokine involved in T cell homeostasis, is also found to be elevated in HIV-1 infected individuals concomitantly with low CD4<sup>+</sup> T cell counts; although the regulation of IL-7 production is not fully understood in the context of HIV-1 infection. Using human intestinal epithelial (DLD-1) and bone marrow stromal (HS-27) cell lines, we investigated in **Paper III**, the consequence of pro-inflammatory cytokines on IL-7 production, measured at the mRNA and the protein levels. Whereas IFN- $\gamma$  induced high IL-7 production in both cell lines, IL-1 $\beta$  treatment led to the opposite effect. We also analyzed the gene expression profiles of HS-27 cells treated with IL-1 $\beta$  and/or IFN- $\gamma$  using the whole-genome microarray Human Gene 1.0 ST. Both cytokines resulted in enhanced expression of genes implicated in T cell immunity, particularly important during HIV-1 pathogenesis. Our results show that the immune activation can lead to profound change in stromal and epithelial cells, which in turn might shape immune responses.

While IL-7 is known to participate to T cell homeostasis, it has recently been shown that this cytokine possibly contribute to B cell defects, leading through IFN- $\gamma$  release by T cells, to Fas up-regulation and sensitivity to Fas-mediated apoptosis. We further evaluated IL-7 regulation of T cell survival in **Paper IV**, and observed that B cells, co-cultured with IL-7 treated T cells, proliferated, displayed a phenotype of differentiated cells and secreted high levels of immunoglobulins (Igs). The Ig secretion was demonstrated to be a consequence of CD70 up-regulation on T cell upon IL-7 treatment. IL-7 led also to BAFF production by T cells, which enhanced B cell survival. In the context of HIV-1 infection, such mechanisms might be implicated in the B cell activation and hypergammaglobulinemia observed in patients.